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ORIGINAL ARTICLE

Fructan distribution in banana cultivars and effect of ripening and processing on *Nendran* banana

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Abstract Many plants store fructan as reserve carbohydrate. Fructans naturally present in almost all plant foods, are also used as functional ingredients by the food industry to modify the texture and taste due to their properties as gelling agents, fat substitutes, soluble dietary fibers and low calorie sweeteners. Seven banana cultivars were analysed for fructans and *Nendran* banana was selected for the next set of experiments as it had the highest fructan content (1433.3 mg/100 g) among the cultivars studied. Low temperature ripening (16 °C) of *Nendran* banana resulted in higher fructan accumulation of these carbohydrates in cold conditions. Pectinase pre-treatment significantly increased yield of total fructans from 1.4/100 g to 6.5 g/100 g i.e., 370 %. Fructan composition was affected by processing, namely steaming and puree preparation in *Nendran*. The fructan composition data documented in this study will enable including banana, naturally high in fructans in the diet and will facilitate storage and processing for nutritional formulation for higher fructan consumption.

Keywords Fructan · *Nendran* banana · Ripening · Processing · Composition

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Introduction

Fructans are oligo and polysaccharides consisting of short chains of fructan units with the single D-glucosyl unit at the reducing end. In humans, there is no enzyme for digesting fructan. Fructans have many health benefits: stimulate the growth of beneficial microorganisms in the gut that limit the pathogens and reduce the risk of colon cancer (Kleessen et al. 2007), significantly increase stool frequency and prevent constipation (Hond et al. 2000), enhance calcium absorption (Roberfroid 2007) and bone mineralization in young adolescents (Abrams et al. 2005), control blood sugar level and insulin requirement (Ayman et al. 2004), reduce plasma levels of cholesterol and triacylglycerol (Beylot 2005) and stimulate the gastrointestinal immune system (Briet et al. 1995).

Fructans exhibit structural diversity in plants. In higher plants, fructans show more differences in fructosyl linkages and are classified into inulin, levan, inulin neoseries, levan neoseries and mixed levan (Vijn and Smeekens 1999). Inulin consists of linear (2–1)-β-D-frutosyl linkages and is present in chicory and Jerusalem artichoke. 1-Kestose or Isokestose is the shortest inulin molecule. Levan consists of linear (2–6)-linked β-D fructosyl units. 6-Kestose is the shortest levan molecule. Mixed type levan consists of both (2–1) and (2–6) β-D-fructosyl units. Inulin neoseries are linear (2–1) β-D-fructosyl units linked to both C1 and C6 of glucose of the sucrose molecule. The shortest molecule of inulin neoseries and mixed type levans is Neokestose. Levan neoseries are polymers of predominantly β (2–6)-linked fructosyl residues on either end of the glucose moiety of the sucrose molecule. Bifurcose is the shortest levan neoseries molecule. Although there are many types of fructosyl residues, in this paper, fructan molecule that consists of β (2–1) residue (1-kestose, nystose and inulin) from different cultivars of banana consumed in South India have been characterized.

India produces 28,455 million tonnes of bananas from an area of 796.5 ha. It is the largest producer of banana not only in Asia but also in the world and contributes 37.2 % to global production followed, by China (6.60 %) and Philippines (6.14 %). TamilNadu contributed 6736.4million tonnes from 130.4 ha with total productivity of 5.17 million tonnes per hectare in the year 2011–12 followed by Maharashtra, Gujarat and Andhra Pradesh (Indian Horticulture Database 2012). There are different cultivars of banana grown in different parts of the world and India grows the largest variety of bananas (20) which are available throughout the year. Banana is a moderate source of fructan (Van Loo et al. 1995). In banana, fructan is synthesized by the action of two different enzymes, 1-SST and 1-FFT. 1-SST transfers fructosyl residue from one sucrose molecule to another sucrose molecule leading to 1-kestose. 1-FFT transfers fructosyl residue from sucrose to 1-kestose thereby elongating the chain leading to 1-nystose. The basic molecule required for synthesizing fructan molecule is sucrose. The demand for fructans, in food industry is increasing steadily because of their functional properties. The objective of the present study was to investigate the distribution of fructans in different banana cultivars and to evaluate the effect of enzymatic treatment and ripening on fructan content from *Nendran* banana. *Nendran* banana was selected as it is the variety used specifically as a weaning food and also commonly processed and consumed. Most other varieties are consumed fresh.

Material and methods

Selection of fruits Seven banana cultivars i.e., *Karpooravalli*, *Morris*, *Rasthali*, *Poovan*, Hill banana, Red banana and *Nendran* used for this study were purchased from local markets and screened for fructans. Each variety was purchased from 10 different locations in and around Chennai city to ensure that variations in their fructan content were represented in the composite sample.

Sample preparation Approximately 500 g of each cultivar from all the 10 outlets was cleaned, washed, peeled, cut and the edible portion taken. Equal edible portions (100 g) from each sample was pooled (10×100 g=1 kg) and thoroughly mixed. From this 1 kg pooled sample, 500 g was blended in a food processor and the homogenized samples were stored frozen in air tight containers at −20 °C until further analysis. From the remaining 500 g, aliquots were used for moisture determination.

Screening and characterization Moisture, titratable acidity and pH were carried out on the same day of sampling. All fruit samples were screened for the presence of total fructans using an enzyme kit. Characterization of fructans was done using HPLC for selected cultivars with high fructan content. All samples were also analysed for sugars (dextrose, fructose and sucrose) by HPLC (O'Donoghue et al. 2004) and inulin content by colorimetric assay (Ashwell 1957).

Ripening of *Nendran* banana The effect of ripening on fructan content was studied in one fruit cultivar *Nendran*. The 1 day old harvested fruits were purchased in bulk from the wholesale fruit market in Chennai and ripened at two different temperatures - room temperature (28±1 °C) and low temperature (16±1 °C).

Two fruits were sampled from storage - daily from the room temperature lot and once in 5 days from the low temperature lot. Samples were blended in a food processor and aliquots of the pulp used for analysing moisture content on the same day. The remaining pulp was stored at −20 °C for further fructan characterization. The ripening study was repeated twice.

Enzymatic pre-treatment In order to study the effect of enzyme treatment on fructan extraction, *Nendran* banana pulp substrate (2 g) was pretreated with four different enzymes independently and also in combination at their respective optimum temperatures - cellulase and hemicellulase at 40 °C;

Table 1 Fructan content of banana cultivars

Banana cultivar	Moisture (%)	pH	Titratable acidity (% anhydrous Citric acid)	Fructan (FW) mg/100 g	Fructan (DW) g/100 g
Hill banana	68.6±0.5	5.3±0.01	0.25±0.02	696.4±2.7	2.22±0.06
Karpooravalli	67.3±1.1	5.6±0.07	0.13±0.03	665.7±23.5	2.04±0.07
Morris	51.7±0.8	5.4±0.04	0.33±0.02	ND	ND
Nendran	55.1±0.3	4.6±0.05	0.42±0.01	1433.3±20	3.19±0.04
Poovan	71.4±0.3	4.5±0.02	0.46±0.03	ND	ND
Rasthali	68.4±0.1	4.8±0.06	0.39±0.33	470.0±37.4	1.49±0.01
Red banana	72.8±1.2	5.2±0.03	0.33±0.01	ND	ND

FW Fresh weight, *DW* Dry weightl, *ND* Not detected

* Moisture, titratable acidity and fructan content, Mean±S.D (n=3)

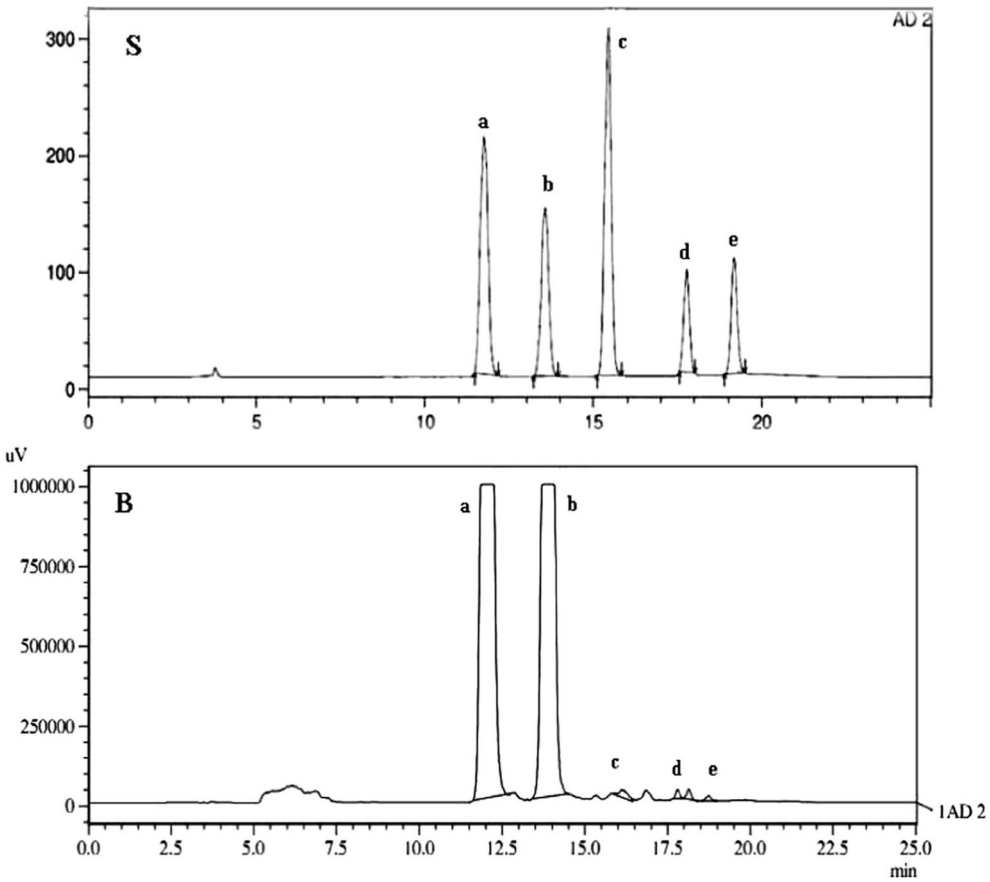

Fig. 1 HPLC-ELSD chromatogram profile for standard (S) & Banana (B) illustrating the location of sugars and fructan fractions in using the Ashahipak NH2P-4E column; with water and acetonitrile as mobile phase. Peaks: **a**, Fructose; **b**, Dextrose; **c**, Sucrose; **d**, 1-kestose; **e**, Nystose

pectinase at 42.5 °C; and invertase at 55 °C for different time intervals of 0, 30, 60 and 90 min.

Steaming One kilogram of *Nendran* cultivar got from one retail shop was divided into two equal portions (500 g each). One portion of the whole fruit was cleaned, peeled and homogenized in a blender and analyzed fresh for its moisture, pH, titratable acidity and fructan content. Another portion of the whole fruit was steam processed in a cooker for 15 min, cooled to room temperature (25 °C) and homogenized in a blender. The homogenized sample was analyzed for its moisture, pH, and titratable acidity on the same day. Both the fresh and steamed portions (each 500 g) were stored in air-tight containers at −20 °C for further analysis.

Puree preparation Two kilograms of *Nendran* got from one retail shop were divided into two portions (500 g and 1.5 kg each). One portion of whole fresh fruit (500 g) was peeled, weighed, homogenized and then analyzed for its moisture, pH, titratable acidity and fructan content. Another portion (1.5 kg) was blanched for 5 min at 80 °C. After cooling, the skin was removed and the fruit was pulped in a homogenizer. Five hundred gram portion was taken and analyzed for its moisture, pH, titratable acidity and fructan content. The puree was prepared from the remaining pulp by heating to increase

Table 2 Sugars in banana cultivars quantified by HPLC

Food	Fructose mg/100 g	Glucose mg/100 g	Sucrose mg/100 g	Kestose mg/100 g	Nystose mg/100 g
Hill banana	54.8±0.9	68.2±1.8	43.0±0.8	17.1±0.7	ND
Karpooravalli	702.8±2.3	5403.4±0.7	856.9±0.7	ND	ND
Nendran	8467.5±1.5	9786.2±1.3	756.2±0.5	61.2±0.4	ND
Rasthali	848.5±1.6	1118.6±1.4	ND	ND	ND

Sugars, Mean±S.D (n=3)

ND Not detected

Table 3 Comparison of FOS content in banana cultivars

Present study (FW) g/100 g					Agopian et al. 2008 (FW) g/100 g		
Banana cultivars	GF_2	GF_3	Inulin	TF	Banana cultivar	GF_2	GF_3
Hill Banana	0.17	ND	0.23	0.69	Ouro	0.29	ND
Karpooravalli	ND	ND	0.17	0.66	Nanicao	0.41	ND
Morris	-	-	-	ND	Prata	0.56	0.03
Nendran	0.07	ND	0.85	0.47	Maca	0.25	ND
Poovan	-	-	-	ND	Mysore	0.55	ND
Rasthali	ND	ND	0.19	1.43	Pacovan	0.44	ND
Red Banana	-	-	-	ND	Terra	0.77	ND
					Figo	0.28	ND

TF present in lower amount than detectable value in banana cultivars. GF_3, GF_4 and Inulin was not analysed in banana cultivars

FW Fresh Weight, *GF_2* 1-kestose, *GF_3* Nystose, *TF* Total fructans, *ND* Not Detected, - Not analyzed

the brix value from 28° to 34° Brix and then cooled to room temperature. Both the fresh pulp and the prepared puree were stored in air tight containers at −20 °C for further analysis.

Analytical methods All reagents used were of analytical grade. Standard sugars like glucose, fructose and sucrose were purchased from Merck, USA and kestose and nystose were purchased from Sigma Aldrich, USA. Enzyme kits for fructan estimation were obtained from Megazyme International Ireland Ltd., Wicklow, Ireland.

Moisture content was determined by oven drying and expressed as percentage. The pH of the diluted sample was determined using a pH meter (Susima A1-plus). Titratable acidity was determined by titrating known amount of the appropriately diluted and filtered sample against standardized sodium hydroxide solution (AOAC 2000) and expressed as anhydrous citric acid percentage. Brix measurement was obtained at a temperature of 28 °C using a portable hand refractometer with the measuring range of 0–32° Brix (Advance Research Instruments, Model: HR-032).

Total fructans The method for extracting fructans fully described in the Megazyme Fructan HK Assay procedure was followed (AOAC 2003). Two samples (A and B) were treated as follows: Sample A was treated with purified fructanase, which hydrolyzed fructan to fructose and glucose, while sample B was treated with blank. The concentration of glucose plus fructose was measured with a hexokinase/phosphoglucose isomerase (PGI) / glucose 6-phosphate dehy-drogenase system. The fructan content was then measured by the difference between sample A and B and expressed on the basis of fresh fruit weight (mg/100 g edible portion) and dry weight (g/100 g edible portion).

Sugar analysis Fruit sample (2 g) was extracted based on the method described by O'Donoghue et al. (2004) with modifications. Distilled water (4.5 mL) was added to the fruit sample and held for 10 min at 75 °C to extract the sugars. To the slurry, 7.5 mL methanol was added to give a final 62.5 %(*v/v*) MeOH solution and extracted for 15 min at 55 °C. The slurry was then passed through a 0.2 μm Millex-GV syringe driven filter. Extracts were stored at −20 °C until further use.

Inulin determination The sample was extracted in 80 % ethanol for 6 h to remove free sugars. The residue was filtered and extracted again in 80 % ethanol for 10 min. The pooled extracts were combined and volume was made-up to 50 mL and analyzed for fructose. The residue was dried and hot water extracted. Inulin content was estimated by using Resorcinol

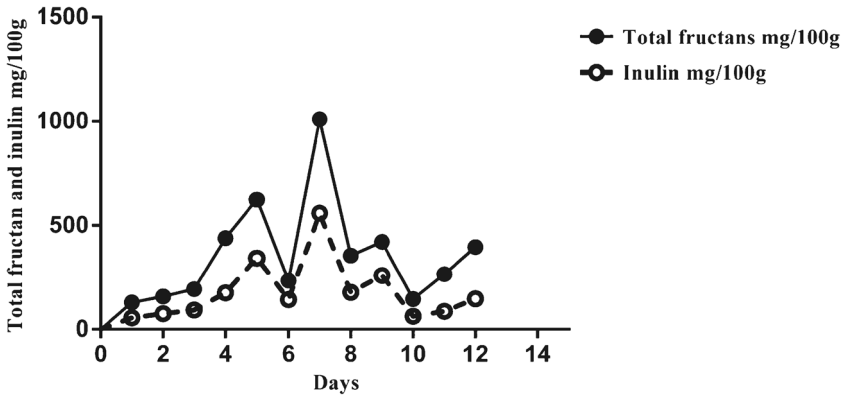

Fig. 2 Total fructan and inulin content of fruits stored at room temperature (28±1 °C)

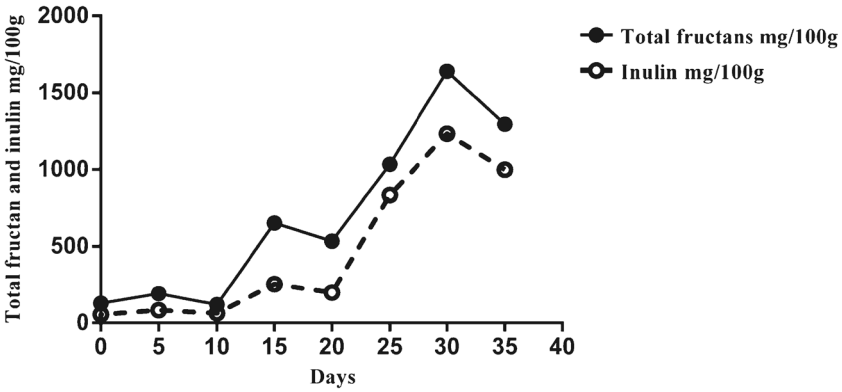

Fig. 3 Total fructan and inulin content of fruits stored at low temperature (16±1 °C)

reagent (Ashwell 1957). Fructose was used to draw the standard graph and the amount of inulin was expressed in terms of g fructose / 100 g edible portion.

Statistical analysis All analyses were carried out in triplicates. Statistical analysis was performed using Graph Pad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The correlation coefficients for enzymatic treatment were analysed by t-test.

Results and discussion

The moisture content of edible portion of banana cultivars ranged from 51.7 to 72.8 % (Table 1). *Nendran* and *Morris* had lower moisture compared to Red banana and *Poovan*. All banana cultivars were acidic (pH between 4.5 and 5.6), *Poovan* being most acidic, while *Karpooravalli* was least acidic (Table 1).

Total fructan was detected in fresh fruit of only four cultivars - *Rasthali*, *Karpooravalli*, Hill banana and *Nendran* (Table 1). *Nendran* contained the maximum fructan, about two to three times of the other cultivars. It is to be noted that all these four varieties are traditionally valued for their health promoting characteristics and *Nendran* and Hill banana are used as weaning food.

Glucose and fructose were the main sugars present in all samples (Fig. 1). Hill banana contained least monosaccharides than other fruits analyzed and is generally less sweet to taste (Table 2). Sucrose was detected in all fruits analyzed, except *Rasthali*. *Nendran* and Hill banana alone were found to contain kestose, while nystose was not detected. Inulin content of these two cultivars was relatively high, with wide variations which were also reflected in total fructan present (Table 3).

Wide differences in FOS content of banana cultivars have been reported by different groups as presented in Table 3. While Campbell et al. (1997) from Ohio reported 1.09 mg/100 g of dry mass, Hogarth et al. (2000) also from Ohio documented 430 and 600 mg/100 g fruit weight at different stages of maturity and Homme et al. (2001) from France reported 130 mg/100 g in banana puree. In contrast, Muir et al. (2007) did not detect fructan in Australian banana. As observed from Table 3, banana cultivars from Brazil had high and varied content of kestose, while nystose was detected only in one cultivar - Prata (Agopian et al. 2008). These differences in the fructan content can be

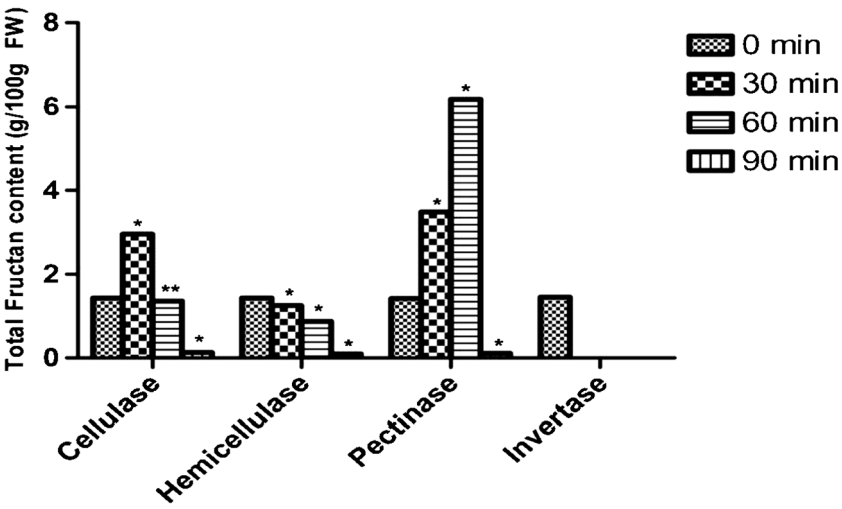

Fig. 4 Effect of enzyme pretreatments on fructan content. * Significantly different when $P<0.05$ from control ** Not significantly different when $P<0.05$ from control

Table 4 Analysis of fresh and steamed fruits (*Nendran*)

	Fresh fruits	Steamed fruits	% Change (+/−)
Moisture content (%)	59.3±0.7	57.6±0.4	−2.9
Titratable acidity (% anhydrous citric acid)	0.43±0.04	0.31±0.02	−27.9
pH	4.6±0.11	4.7±0.26	+2.1
Fructan content (FW) mg/100 g	1494.7±22.5	1158.0±9.0	−22.5
Fructan content (DW) g/100 g	3.7±0.05	2.7±0.01	−25.7

FW FreshWeight, *DW* Dry Weight

* Mean±S.D (n=3)

attributed to several factors such as soil, cultivar, stage of ripening and storage conditions.

Effect of ripening in *Nendran*

All analyzed results are the means of duplicate sets of *Nendran* banana stored at 16±1 and 28±1 °C. In the first batch of fruits ripened at 16 °C, fructan peaked on the 25th day, while in the second it peaked on the 30th day. In contrast at 28 °C, the peak fructan was observed on the 7th day in both batches. The pulp to peel ratio increased steadily in fruits stored at both room (28±1 °C) and low (16±1 °C) temperature whereas, the moisture content decreased and then increased during the final stages of ripening (Data not shown).

Total fructan content of *Nendran* banana attained its peak value at day-7 in case of room temperature storage (28 °C) and day-30 in case of low temperature (16 °C) (Figs. 2 and 3). In low temperature storage, all analyzed parameters were delayed by 15 days. Gradual increase in fructans was noted during ripening at both temperatures. Both fructan and inulin levels accumulated significantly (p<0.05) by 58.5 and 29.4 % respectively, during ripen-ing at low temperature, compared to room temperature storage. All sugars accumulated to a larger extent at low temperature than at room temperature - fructose and glucose by 17.4 and 59.4 % respectively; 1-kestose by 33.4 % and sucrose by 25.6 % (Data not shown).

The results of the present study are similar to the results published by Agopian et al. (2009). However differences in cultivars, degree of ripening, and the presence of fructan outside the inulin neo series could explain the discrepancies reported. The results also suggest that the start of 1-kestose accumulation is highly dependent on the specific amount of sucrose accumulated since kestose was not present in the unripe banana.

Effect of enzymatic pretreatment in *Nendran*

Pectinase pretreatment alone significantly increased total fructans from initial 1.4/100 to 6.5 g /100 g i.e., 370 % when compared to other enzymes (Fig. 4). The degradation of pectic substances, which are predominant structural constituents of primary cell wall, could explain this. They are the sole polysaccharides in middle lamella, along with some cellulose micro fibrils, while they may be virtually absent in secondary

Table 5 Analysis of fresh fruits and fruit puree (*Nendran*)

		Fresh fruit	Blanched fruit	Fruit puree
Moisture content (%)		51.87±0.49	54.83±0.59	43.86±0.09
Titratable acidity (% anhydrous citric acid)		0.64±0.03	0.68±0.02	0.68±0.01
pH		4.2±0.14	4.19±0.26	4.17±0.21
Fructan content (FW) mg/100 g		1493.7±0.29	1287.6±0.51	2471.1±0.77
Inulin mg/100 g		870.4±0.68	976.6±0.39	1025.2±0.78
Colour	L*	62.5±0.03	64.2±0.17	58.9±0.09
	A*	10.8±0.02	10.5±0.14	10.2±0.21
	B*	31.5±0.08	25.4±0.12	28.4±0.03
Texture	Firmness (g)	308.6±0.27	301.5±0.16	342.6±0.19
	Consistency (gs)	9224.0±0.15	9237.3±0.17	9382.6±0.18
	Cohesiveness (g)	182.0±0.17	180.9±0.06	198.2±0.15
	Index of viscosity (gs)	7320.0±0.09	7314.7±0.04	8010.4±0.13

FW FreshWeight

* Mean±S.D (n=3)

walls. Cellulase, hemicellulase and pectinase combined pretreatments increased total fructans from initial 1.4/100 to 6.2 g /100 g i.e., by 334 %. Evidently, single cell wall degrading enzyme, pectinase could enhance fructan extraction significantly than the combination.

Pectinase treatment caused reduction in fructose content, while sucrose showed a slight increase. Glucose did not change whereas; slight decrease in kestose was observed. Inulin content of the fruit increased from initial 850.2 to 2780.3 mg / 100 g corresponding to an increase of 227 %. Our experiments with enzyme-aided extraction of fructan from *Nendran* demonstrate the potential for recover-ing natural prebiotic fructan effectively from other fruits.

Effect of steam processing in *Nendran*

Moisture content and titratable acidity decreased for steamed fruits, where as pH increased. Steaming decreased acidity and fructan in *Nendran* (Table 4). The loss in fructan observed in the present study is similar to the result reported by Hogarth et al. (2000), who documented a decrease in fructan content in processed banana, grapes and tomato.

Effect of puree processing in *Nendran*

Moisture content of *Nendran* decreased from 51.8 to 43.8 %, titratable acidity increased slightly, and pH decreased on pureeing. Total fructan and inulin increased in puree due to its concentration/loss of water (Table 5). The increase in fructan content observed in the present study is different from the result reported by Homme et al. (2003), who observed that fructan loss was insignificant at 80–100 °C for 30 min in apple dessert, apple puree, banana puree and stewed apple-banana fruits.

L*, a* and b* value showed that colour was brighter in fresh fruit compared to puree. *Nendran* puree showed more firmness, consistency and cohesiveness compared to fresh fruits.

Conclusion

Fructan composition in banana is influenced by the cultivar and varies in the levels of low molecular forms and inulin. Low temperature ripening (16 °C) of *Nendran* banana resulted in higher fructan distribution indicating accumulation of these carbohydrates in cold conditions. Treatment of *Nendran* banana with pectinase enhanced fructan extraction significantly. The relatively simple enzymatic method developed could provide a basis to optimize extraction from other food sources. Fructan loss during steaming of *Nendran* banana may be due to thermal degradation. In contrast, fructan content increased during puree preparation because of the concentration resulting from loss of moisture. The fructan data documented in this study will facilitate incorporation of high-fructan natural foods in the diet and nutritional formulation and gourmet selection for optimal fructan consumption.

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